UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	**February 21, 2002**
LISZIEWICZ, et al.	:	**Atty Docket No. RGT 9771**
DIVISION OF **Serial No. 09/153,198**	:	**Group 1633**
Filed: 15 September 1998	:	**Examiner: Wilson**

For: Method of Delivering Genes into Antigen Presenting Cells of the Skin

Commissioner for Patents
And Trademarks
Washington, D.C. 20231

Fax: (703)-308-8724 Phone (703) 308-0120[1]

Preliminary Amendment for Divisional Application

In the Claims,

Please cancel Claims 1-22.

Please add Claims 23-41

23. A method of transducing antigen-presenting cells of the skin, the steps comprising selecting a gene delivery complex comprising DNA and a compound selected from the group consisting of sugars, polyethylenimine, polyethylenimine derivatives, and mixtures thereof,
and applying the complex to the skin or mucosa surfaces of an animal,
wherein said DNA comprises a nucleic acid sequence encoding at least one immunogenic protein operatively linked to a promoter.

24. The method of Claim 23, wherein the compound is selected from the group consisting of glucose and polyethylenimine derivatives.

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[1] This paper is being forwarded to the Commissioner for Patents and Trademarks, Washington D.C., 20231 by Express mail label No. EG636908054 on February 21, 2002 by Valerie E. Looper, signed Valerie E. Looper.

25. The method of Claim 24, wherein the polyethyleninine derivative targets the mannose receptor found on the suface of antigen presenting cells.

26. The method of Claim 25, wherein the derivative is mannosylated polyetheylenimine.

27. The method of Claim 26, wherein the mannosylated polyethylenimine is derived from a linear PEI 22 kDA.

28. The method of Claim 23, wherein the complex is electrostatically neutral. (page 25, lines 9-32).

29. The method of Claim 23, wherein the complex comprises about 3-10:1 molar equivalent polyethylenimine or polyethylenimine derivative amine per DNA phosphate ratio. (page 25, lines 26-27, page 24).

30. The method of Claim 23, wherein the complex comprises about 5:1 molar equivalent polyethylenimine or polyethylenimine derivative amine per DNA phosphate ratio. (page 25, lines 26-27, page 24).

31. The method of Claim 23, wherein the gene delivery complex is formulated in a glucose solution.

32. The method of Claim 31, wherein the glucose solution is about 5-10% glucose.

33. The method of Claim 32, wherein the glucose solution is about 8% glucose.

34. The method of Claim 23, further comprising the step of activating the antigen presenting cells of the skin or mucosa surfaces of the animal.

35. The method of Claim 34, wherein the activating step is performed by receptor stimulation, toxin activation, or tissue or cell injury.

36. The method of Claim 23, wherein the immunogenic protein is derived from a reverse-transcriptase dependent virus.

37. The method of Claim 36, wherein the reverse transcriptase-dependent virus is a human immunodeficiency virus.

38. The method of Claim 37, wherein the human immunodeficiency virus is replication-defective.

39. The method of Claim 38, wherein the human immunodeficiency virus is integration-defective.

40. The method of Claim 23, wherein the DNA is a plasmid.

41. The method of Claim 23, wherein the cells are Langerhans cells.

Remarks

New Claims Nos. 23-41, based on original Class II of parent application USSN 09/153,198, which had been subject to a restriction requirement, are presented. These Claims are supported by the original Claims. The specification has been converted to another electronic format, repaginated, and checked for obvious typographical errors; changes made to the specification have been cumulated. Mark-up and clean copies of the paragraphs or sections of the application are enclosed, as well as a clean copy of the Claims.

A copy of the Specification, Figs 1-7, the Oaths and Declarations and Verified Statements of Small Entity status from the original application are enclosed, together with PTO forms 1449 and 892. If the Examiner wishes, the Applicants will forward copies of the references cited therein.

New form 1449, and a copy of USPN 5,916,803 to Sedlacek, are enclosed. This reference is not believed to be closer than the cited art, because it relates to a nonviral vector including a fusion protein.

Respectfully Submitted,

Valerie E. Looper
Registration No. 33,007

The Law Offices of
Valerie E. Looper
11726 Lightfall Court
Columbia, Maryland 21044
Telephone: (410) 715 - 5771
fax: (410) 715-5773

CLEAN VERSION OF THE REPLACEMENT PARAGRAPHS/SECTIONS

In the Specification

At page 2, the title "Method of Delivering Genes into Antigen Presenting Cells of the Skin".

At page 2, add a new section before the "Field of the Invention"

Related Application Information

This application is a division of USSN 09/153,198, which is a continuation-in-part of USSN 60/058,933, filed September 15, 1997, both of which are incorporated herein as if set forth in full.

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There is some evidence suggesting that genetic modification of APC will be effective to vaccinate both neonatal and adult animals and humans. Ridge et al. (Science 271: 1723-1726, 1996) have injected DC expressing a foreign antigen isolated from another animal intravenously into mice. Both neonatal and adult mice injected with these DC were able to generate good CTL killing of target cells. These experiments also demonstrated that DC expressing a foreign antigen can induce protective cell-mediated immune responses which is able to eliminate infected cells in case of viral infections. In addition, these experiments demonstrated that DC-mediated immunization of neonates may be possible. These experiments did not use genetically modified cells, nor did they utilize several foreign antigens nor a virus as described in the present invention.

At page 6, line 33

Fig. 7A-7D is a color photograph showing that transcutaneous genetic immunization results in gene expression in the lymphoid organs.

At page 7, line 18

In Fig. 3, the experiment demonstrating in vivo the sugar-mediated gene delivery into cells expressing mannose-receptors is illustrated. Target cells are Langerhans cells in the skin, which are known to express mannose receptors. Mice (21) were anesthetized and an area on the back of each mouse (22) was shaved. The shaved surface was cleaned with ethanol. The PEI-man-DNA gene delivery complex in 8% glucose (23) was applied to the shaved area (22) of each mouse. Langerhans cells (24) found in the shaved area of the skin (22) pick up the complex as described in Fig. 2 above, get activated and migrate

(24) to the draining lymph node (25). During the migration Langerhans cells (24) mature into dendritic cells (26) and express the protein (27) encoded by the DNA.

At page 8, line 1,

Fig. 7A-7D reports experimental evidence that transcutaneous transduction of Langerhans cells results in migration of the cells and expression of the transferred gene. The figure is a series of color photographs which records green cells having DC morphology and expressing the green fluorescent protein, which is the product of the gene which was transferred via skin delivery. Panel A is a sample of a lymph node from a control mouse at 200x magnification. It exhibits a normal amount of background fluorescence. The same is true of Panel C, except that the magnification is 400x. Panel B is a sample from a lymph node of a mouse that was immunized by the transcutaneous application of a PEI-mannose-DNA complex. Panel D is the same as Panel B, except that the magnification is 400x. The fluorescence exhibits the bumpy morphology characteristic of dendritic cells expressing proteins.

At page 8, line 24,

An advantage of the present invention is that it provides a in vivo gene transfer method which can be utilized for immunotherapy and vaccination for a wide variety of diseases. Another advantage of the present invention is that it can utilize any type of DNA, or RNA, including plasmid DNA encoding immunogens like oncogens, immunogens (causing allergy), viral proteins or different types of replication defective viruses, defective viral particles, as well as plasmid DNA. Another advantage of the present invention is that it can utilize instead of DNA proteins like oncogenic protein (e.g. mutated p53 or Ras), immunogens (causing allergy), viral proteins or different types of replication defective viruses.

At page 13, line 26,

The choice of the gene delivery particle will be determined by the disease and the choice of gene(s) to transfer. Where it is desired to construct a vaccine for a reverse-transcriptase dependent virus such as HIV, the DNA preferably encodes at least a

substantial portion of a replication-or integration defective virus or the replication- or integration-defective virus itself. Examples include but are not limited to integrase negative mutants of a dual-tropic primary isolate such as HIV-1/LW, and derivatives thereof having a deletion in the protease cleavage site of the gag gene or where the DNA further includes one or more stop codons in one or more reading frames of the integrase gene. See Methods and Compositions for Protective and Therapeutic Genetic Immunity, USSN 08/803,484 filed Feb. 20, 1997 and incorporated by reference as if set forth in full. Where it is desired to construct a vaccine for cancer, the immunogen is preferably DNA encoding one or more oncogens. Other DNA constructs can be DNA encoding replication defective Human Papilloma Virus (causing cervical cancer), replication defective Hepatitis A, B and C viruses (causing hepatitis and liver cancer), and DNA encoding replication defective animal viruses like Bovine Leukemia Virus or Feline Immunodeficiency Virus. Choices for a delivery particle incorporating the foreign genetic material can include: (a) replication defective HIV or other retrovirus; (b) recombinant adenovirus; (c) plasmid or linear DNA or RNA complexed with PEI or a derivative of PEI; (d) a virosome containing any DNA or RNA; (e) liposome containing DNA or RNA; (f) plasmid DNA-polylysine virus complex; (g) sugar complexed with any DNA or RNA.

At page 14, line 28

Non-viral gene delivery systems offer several advantages over viral gene delivery systems: 1) First, the non-viral vector is not recognized by the immune system, so no immune response is generated against it. As a result, it is more likely that individuals treated with the ultimate vaccine will tolerate and develop adequate immune response in cases of repeated immunization; 2) non-viral systems are potentially more safe that viral systems because there is no possibility that the system will mutate in an unexpected fashion; 3) non-viral systems can be chemically synthesized in a large amounts, and are therefore potentially less expensive.

At page 15, line 16,

The mannose receptor is a 175-kDa transmembrane glycoprotein that specifically expressed on the surface of macrophages and Langerhans cells. The ectodomain of the mannose receptor has eight carbohydrate recognition domains. The mannose receptor recognizes the patterns of sugars that adorn a wide array of bacteria, parasites, yeast, fungi, and mannosylated ligands. [Takahashi K; Donovan MJ; Rogers RA; Ezekowitz RA, Cell Tissue Res 1998 May; 292(2):311-23]. In contrast to the Fc receptor, the mannose receptor reconstitutes itself while releasing its cargo [Stahl et al. Cell 1980 19:207]. It thus can internalize of ligands in successive rounds, in a manner similar to the transferrin receptor, providing a sustained capacity for antigen capture[Goldstein, et al, 1985, Annu Rev Cell Biol. 1:1]. It has been recently discovered that mannose-receptor-mediated uptake of antigens results in about 100 fold more efficient antigen presentation to T-cells, as compared to antigens internalized via fluid phase [Engering et al. 1997, Eur. J. Immunol. 27:2417-2425]. This enhanced antigen presentation is due to highly efficient uptake of antigens via the mannose receptor. For these reasons we believed that targeting the mannose receptor may yield both specificity for antigen presenting cells and improved efficiency of functional uptake of the complex into the endosome.

At page 17, line 29,

Drug combinations that are effective to at least temporarily inhibit HIV replication are known. The inventors have shown that drug combinations including hydroxyurea, one or more reverse transcriptase inhibitors and, optionally, one or more protease inhibitors are particularly effective, and, for some patients, allow the possibility of stopping drug treatment for extended periods of time. See USSN 09/056,691, filed Apr. 8, 1998 "Method of Inhibiting HIV by Combined Use of Hydroxyurea, a Nucleoside Analog, and a Protease Inhibitor, USSN 09/048,886 filed Mar. 26, 1998 Method of Inhibiting HIV using Hydroxyurea and Reverse Transcriptase Inhibitor in vivo and USSN 09/048,576, filed March 26, 1998, Method of Rendering a HIV Population Replication Incompetent in vivo, all of which are incorporated herein by reference as if set forth in full. The present

invention includes the treatment of a patient with active HIV infection with an appropriate drug combination until the viral load in the blood has reached a suitably low level, less than about 50,000 copies per ml, preferably less than 10,000 copies per ml, more preferably less than 200-500 copies per ml. The patient is then vaccinated using the present invention while the drug combination suppresses replication of the wild type virus.

At page 18, line 26,

Monocyte-derived DC were generated from peripheral blood mononuclear cells in the presence of GM-CSF and IL-4. [Bender, A., M. Sapp, et al. (1996). "Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood." J Immunol Methods 196(2): 121 35.] On day 4, cells were transfected with lipofectamine complexed with plasmid DNA encoding HIV-1/LWint- (an integration and replication defective HIV described in USSN 08/803,484). Lipofectamine, a commercially available cationic liposome useful as a transfection reagent (available from Gibco BRL Life Technology, PM. Gaithersburg, Md., US). 48 hours later, the cells were washed and analyzed. The purity of the DC, characterized by Fluorescence Activated Cell Sorter (FACS) measuring surface markers (FACS) was 90.6%. DC cell types were found to be CD3-, CD19-, CD56-, CD14- and HLA DR+ using FACS analysis. The expression of HIV-1 Gag and Env and Tat proteins was also measured by FACS in permeabilized cells. The level of non-specific binding of isotope control Ig was the same in the control transduced and specific plasmid transduced. We found in three independent experiments that 25-37% of HIV-1/LWint-transduced DC expressed Env, Gag and Tat proteins. That is, 25-37% of the cells in the transduced samples expressed HIV proteins. Transduced and control cell samples were also double-stained with p24 and B7-2 antibodies to demonstrate that DC and not macrophages were expressing the antigen. These results were surprisingly good, because using the same methods with another plasmid DNA (CMV-driven hemagglutinin of influenza virus gene) only 5-8% of the transfected cells

expressed proteins. These results demonstrated that defective HIV can be efficiently expressed by transduced DC.

At page 19, line 14,

2. DNA encoding replication defective viruses are more efficient antigens than DNA encoding one or more proteins

In an independent experiment we compared the expression of two different HIV plasmids in DC: HIV-1/LWint- and LTR-tat. Both constructs are driven by the same promoter: HIV-1-LTR and the expression of both constructs depends on the transactivation of Tat. Transfection was performed as described in example 1 and 48 hours later expression of the Tat protein was analyzed by FACS. We found that 32% of HIV-1/LWint- plasmid transfected DC expressed Tat protein. In contrast only 10% of the LTR-tat transfected DC expressed the same Tat protein. This result was surprising, because in this comparative study we were expected the same efficiency with the two different constructs. Replication defective viruses definitely have the capability to form viral particles, which can be released from the cell. Since antigen presentation depends on gene expression in DC, this experiment clearly demonstrates that DNA encoding defective viruses are more efficient antigens than DNA encoding one or more proteins.

at page 20, line 20

5. Polyethyleneimine-mediated Gene Transfer into Cultured Dendritic Cells.

Dendritic cells were transduced with plasmid encoding HIV-1/LWint- as in Example 1, except that polyethylenimine (PEI kindly supplied by Dr. Behr) was used instead of lipofectamine. The cells were tested as in Example 1, and more than 60% of the dendritic cells transduced using polyethylenimine expressed HIV-1 proteins in contrast to the 25-37% of cells transduced using lipofectamine. Since to date lipofection was the best gene transfer method to introduce plasmid DNA into DC, this experiment demonstrates that PEI is the most efficient non viral gene delivery system to transfer genes into DC. However, both PEI and lipofectamine exhibited significant toxicity to dendritic cells, as measured by tripan blue staining.

At page 21, line 8,

PEI modified with different sugars was chosen to target the mannose receptor on the surface of dendritic cells because the mannose receptor recognizes all the patterns of sugars on the surface of bacteria, parasites, yeast and fungi. DNA was complexed with PEI and with different sugar-bearing polyethylenimines (available on a custom order basis from Dr. Jean-Paul Behr, Laboratoire de Chimie Genetique, Faculte de Pharmacie, CNRS-UMR 7514 74 route du Rhin 67401 Illkirch, France). 2 microgram DNA was incubated with different derivatives of PEI in 150mM NaCl (10:1 N:P ratio) at room temperature for about 5 minutes. Then DC were transduced with the complexes for 6 hours washed, and green fluorescent cells were analyzed after 48 hours. We found that the most effective PEI-sugar modification is the PEI-mannose (Table 1).

At page 22, first partial paragraph,

neutralized by the DNA, the complex cannot enter via the asialoglycoprotein receptor. [Zanta MA; Boussif O; Adib A; Behr JP. Bioconjug Chem 1997 Nov-Dec;8(6):839-44]: These investigators developed a hepatocyte-directed complex; it includes several key features thought to favor in vivo gene delivery to the liver: 1) electrostatically neutral particles which avoid nonspecific binding to other cells, and 2) to avoid asialoglycoprotein receptor-mediated endocytosis. This system was based on a 5% galactose-bearing polyethylenimine (PEI-gal) polymer which is condensed with plasmid DNA to neutrality.

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At page 2, add a new section before the "Field of the Invention"

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At page 2, delete the first sentence of "Field of the Invention"

~~This application is a continuation-in-part of USSN 60/058,933, filed September 15, 1997, which is incorporated herein as if set forth in full.~~ The present invention relates generally to methods and compositions for delivering foreign genetic material into cells. Specifically, it relates to a technique for receptor-mediated delivery of genes to cells. A gene delivery complex compatible with a specific type of targeted cell is formed from the foreign genetic material, a vector, and optionally, a carrier. The complex is then exposed to the cells under conditions permitting receptor-mediated endocytosis, resulting in the functional uptake, or transduction, of the foreign genetic material. The method is not only useful for in vitro, but also in vivo gene delivery to antigen presenting cells, specifically described as transcutaneous gene transfer to skin Langerhans cells. This technique is particularly useful for preventive and therapeutic genetic immunization when the foreign genetic material is an immunogen such as DNA encoding a substantial portion of the antigens and particles associated with an infectious virus, and where delivery by injection is undesirable.

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Non-viral gene delivery systems offer several advantages over viral gene delivery systems: 1) First, the non-viral vector is not recognized by the immune system, so no immune response is generated against it. As a result, it is more likely that individuals treated with the ultimate vaccine will tolerate and develop adequate immune response in cases of repeated immunization; 2) non-viral systems are potentially more safe that viral systems because there is no possibility that the system will mutate in an unexpected fashion; 3) non-viral systems can be chemically synthesized in a large amounts, and are therefore potentially less expensive.

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48 hours later, the cells were washed and analyzed. The purity of the DC, characterized by Fluorescence Activated Cell Sorter (FACS) measuring surface markers (FACS) was

90.6%. DC cell types were found to be CD3-, CD19-, CD56-, CD14- and HLA DR+ using FACS analysis. The expression of HIV-1 Gag and Env and Tat proteins was also measured by FACS in permeabilized cells. The level of non-specific binding of isotope control Ig was the same in the control transduced and specific plasmid transduced. We found in three independent experiments that 25-37% of HIV-1/LWint-transduced DC expressed Env, Gag and Tat proteins. That is, 25-37% of the cells in the transduced samples expressed HIV proteins. Transduced and control cell samples were also double-stained with p24 and B7-2 antibodies to demonstrate that DC and not macrophages were expressing the antigen. These results were surprisingly good, because using the same methods with another plasmid DNA (CMV-driven hemagglutinin of influenza virus gene) only 5-8% of the transfected cells expressed proteins. These results demonstrated that defective HIV can be efficiently expressed by transduced DC.

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2. DNA encoding replication defective viruses are more efficient antigens than DNA encoding one or more proteins

In an independent experiment we compared the expression of two different HIV plasmids in DC: HIV-1/LWint- and LTR-tat. Both constructs are driven by the same promoter: HIV-1-LTR and the expression of both constructs depends on the transactivation of Tat. Transfection was performed as described in example 1 and 48 hours later expression of the Tat protein was analyzed by FACS. We found that 32% of HIV-1/LWint- plasmid transfected DC expressed Tat protein. In contrast only 10% of the LTR-tat transfected DC expressed the same Tat protein. This result was surprising, because in this comparative study we were expected the same efficiency with the two different constructs. Replication defective viruses definitely have the capability to form viral particles, which can be released from the cell. Since antigen presentation depends on gene expression in DC, this experiment clearly demonstrates that DNA encoding defective viruses are more efficient antigens than DNA encoding one or more proteins.

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PEI modified with different sugars was chosen to target the mannose receptor on the surface of dendritic cells because the mannose receptor recognizes all the patterns of sugars on the surface of bacteria, parasites, yeast and fungi. DNA was complexed with PEI and with different sugar-bearing polyethylenimines (available on a custom order basis from Dr. Jean-Paul Behr, Laboratoire de Chimie Genetique, Faculte de Pharmacie, CNRS-UMR 7514 74 route du Rhin 67401 Illkirch, France). 2 microgram DNA was incubated with different derivatives of PEI in 150mM NaCl (10:1 N:P ratio) at room temperature for about 5 minutes. ~~Than~~ Then DC were transduced with the complexes for 6 hours washed, and green fluorescent cells were analyzed after 48 hours. We found that the most effective PEI-sugar modification is the PEI-mannose (Table 1).

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neutral particles which avoid nonspecific binding to other cells, and 2) to avoid asialoglycoprotein receptor-mediated endocytosis. This system was based on a 5% galactose-bearing polyethylenimine (PEI-gal) polymer which is condensed with plasmid DNA to neutrality.

Clean version of the Claims

23. A method of transducing antigen-presenting cells of the skin, the steps comprising selecting a gene delivery complex comprising DNA and a compound selected from the group consisting of sugars, polyethylenimine, polyethylenimine derivatives, and mixtures thereof,
and applying the complex to the skin or mucosa surfaces of an animal,
wherein said DNA comprises a nucleic acid sequence encoding at least one immunogenic protein operatively linked to a promoter.

24. The method of Claim 23, wherein the compound is selected from the group consisting of glucose and polyethylenimine derivatives.

25. The method of Claim 24, wherein the polyethyleninine derivative targets the mannose receptor found on the suface of antigen presenting cells.

26. The method of Claim 25, wherein the derivative is mannosylated polyetheylenimine.

27. The method of Claim 26, wherein the mannosylated polyethylenimine is derived from a linear PEI 22 kDA.

28. The method of Claim 23, wherein the complex is electrostatically neutral. (page 25, lines 9-32).

29. The method of Claim 23, wherein the complex comprises about 3-10:1 molar equivalent polyethylenimine or polyethylenimine derivative amine per DNA phosphate ratio. (page 25, lines 26-27, page 24).

30. The method of Claim 23, wherein the complex comprises about 5:1 molar equivalent polyethylenimine or polyethylenimine derivative amine per DNA phosphate ratio. (page 25, lines 26-27, page 24).

31. The method of Claim 23, wherein the gene delivery complex is formulated in a glucose solution.

32. The method of Claim 31, wherein the glucose solution is about 5-10% glucose.

33. The method of Claim 32, wherein the glucose solution is about 8% glucose.

34. The method of Claim 23, further comprising the step of activating the antigen presenting cells of the skin or mucosa surfaces of the animal.

35. The method of Claim 34, wherein the activating step is performed by receptor stimulation, toxin activation, or tissue or cell injury.

36. The method of Claim 23, wherein the immunogenic protein is derived from a reverse-transcriptase dependent virus.

37. The method of Claim 36, wherein the reverse transcriptase-dependent virus is a human immunodeficiency virus.

38. The method of Claim 37, wherein the human immunodeficiency virus is replication-defective.

39. The method of Claim 38, wherein the human immunodeficiency virus is integration-defective.

40. The method of Claim 23, wherein the DNA is a plasmid.

41. The method of Claim 23, wherein the cells are Langerhans cells.